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Effects of Short-term High-carbohydrate Feeding on Serum Triglyceride of Children with Familial Hypercholesterolaemia*

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Segall, M. M., Tamir, I., Fosbrooke, A. S., Lloyd, J. K., and Wolff, O. H. (1970). *Archives of Disease in Childhood*, **45**, 393. **Effects of short-term high-carbohydrate feeding on serum triglyceride of children with familial hypercholesterolaemia.** In 5 children with familial hypercholesterolaemia, serum triglyceride levels, which were initially normal, rose after three days on a high-carbohydrate diet; a similar response occurred in one child with normal serum lipoproteins. These observations suggest that a rise in serum triglyceride on high-carbohydrate feeding is a normal finding in children and therefore should not be used as a test for the diagnosis of the pathological state of 'carbohydrate-induced hypertriglyceridaemia'. The findings in familial hypercholesterolaemia indicate that diets used in the treatment of this condition should not contain an unduly high proportion of carbohydrate.

Detailed investigations showed that most of the increase in triglyceride occurred in the very low density lipoproteins, but small increases were also found in the other lipoproteins. The lipid composition of all the lipoproteins changed, the proportion of triglyceride being increased. In all children the fatty acid composition of serum triglyceride (g./100 g. total fatty acids) showed an increase in palmitoleic acid and a decrease in linoleic acid. A raised percentage of palmitoleic acid appears to be the most consistent indicator of accelerated lipogenesis during high-carbohydrate feeding. Changes in triglyceride fatty acid composition were similar in all the lipoproteins. On high-carbohydrate feeding the absolute concentration of all triglyceride fatty acids, including linoleic acid, increase.

High-carbohydrate diets have been shown to increase the serum triglyceride levels in healthy adults during long- and short-term feeding periods (Antonis and Bersohn, 1961; Lees and Fredrickson, 1965; McGandy, Hegsted, and Stare, 1967), but the effects of such diets have not been described in children. In the dietary treatment of familial hypercholesterolaemia in children, a considerable reduction in saturated fat intake is necessary (Segall, 1968), and, unless sufficient polyunsaturated fat is added, the calorie requirements have to be made up mainly by carbohydrate. As part of our studies on the dietary treatment of this condition we have observed the effects of a short-term high-carbohydrate diet on the serum triglyceride of 5 children with familial hypercholesterolaemia. We have also observed the effects of the diet on a child with normal serum lipoproteins; since all our subjects had normal triglyceride levels, the study may also provide some evidence on the normal response of serum triglyceride to high-carbohydrate feeding in childhood.

Subjects and Methods

Subjects. The age and sex of the children are given in Table I. The 5 children with familial hypercholesterolaemia had the heterozygous form of the disease (range of serum cholesterol 306–434 mg./100 ml.). Case 6 was in hospital for treatment of chronic constipation; he and his parents agreed to co-operate in our investigation. None of the children was obese.

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Diets. An assessment of the children's usual dietary intake was made. All the diets in hospital were designed to be isocaloric with the home diets and were given as normal foods. In the high-carbohydrate diets the extra carbohydrate was given in place of fat, and 75% of the total calories were derived from carbohydrate (of which 75% was sucrose), 10% from fat, and 15% from protein. The children took the diets well and their carbohydrate intake is given in Table I. In most children the body weight remained constant; Case 3 lost 1·4 kg. and Case 6 gained 1·2 kg.

In the hypercholesterolaemic children the high-carbohydrate diet was continued for 10 days and in the normolipidaemic child for 6 days. After this period the diets of the hypercholesterolaemic children were changed isocalorically to contain 45% of calories from carbohydrate, 40% from fat (mainly in the form of corn oil), and 15% from protein.

Analytical methods. Venous blood, obtained after an overnight fast, was taken from all subjects at the start of, and after 3 and 6 days on, the high-carbohydrate diet. In some children samples were also taken after 10 days on the diet, and 1 and 7 days after its discontinuation.

Serum lipoproteins were studied using paper electrophoresis (Salt and Wolff, 1957); preparative ultracentrifugation in a saline density gradient (Cornwell *et al.*, 1961) was used to separate three classes of lipoproteins: very low density (VLDL, density 1·023 g./ml.), low density (LDL, density 1·036 g./ml.), and high density (HDL, density 1·070 g./ml.). In order to exclude any residual dietary triglyceride in the form of chylomicrons, ultracentrifugation was preceded by centrifugation of the serum at 10,000 r.p.m. (8000 × *g*) for 30 minutes, the surface layer being discarded. When the children were taking their usual diet (approximately 40% calories from fat and 45% from carbohydrate), the quantity of triglyceride removed by this procedure was small (mean 5 mg./100 ml. serum). However, on the high-carbohydrate diet (10% calories from fat and 75% from carbohydrate) appreciable amounts of triglyceride were removed (mean 50 mg./100 ml. serum) We have assumed that this triglyceride was of endo genous origin present in VLDL, because at this time th dietary fat was low, the serum was optically clear, an no chylomicron band was seen on paper electrophoresis We have therefore calculated the triglyceride concentra tion in VLDL by subtracting the triglyceride present i LDL and HDL from that in the whole serum.

Serum triglyceride concentration and fatty acid com position were estimated by gas-liquid chromatograph (Fosbrooke and Tamir, 1968), serum total cholesterol b an Autoanalyser method (Technicon method N-24a),* and serum phospholipid (lipid phosphorus × 25) by a modification of the method of Bartlett (1959).

Results

Table I shows the effect of high-carbohydrat diet on the serum triglyceride levels. In al children before introduction of the diet the serun triglyceride was normal (within the range 28–8 mg./100 ml. found in 11 healthy subjects; 4 childre and 7 adults under 30 years of age). In each cas serum triglyceride increased after 3 and 6 days o the diet, and in the 3 hypercholesterolaemic childre examined after 10 days levels were still raised The normolipidaemic boy (Case 6) showed increase similar to those found in the hypercholesterolaemi children. The maximum value observed varie considerably in the individual children (50–29 mg./100 ml.); it was not related to age.

In all 5 hypercholesterolaemic children serun triglyceride levels fell markedly on resumption of a diet containing 45% of calories from carbohydrate the fall was already apparent after one day in th four children examined at this time.

The triglyceride content of the lipoprotei fractions is shown in Table II. The major increas in triglyceride on high-carbohydrate feeding occur-

*Technicon Instruments Ltd., Chertsey, Surrey, England.

TABLE I

Effect of High-carbohydrate Diet on Serum Triglyceride Levels

Case No.	Sex	Age (yr.)	Mean Carbohydrate Intake (g./day)		Mean Carbohydrate Intake (g./kg. per day)		Serum Triglyceride mg./100 ml.					
							Days on High-carbohydrate Diet				Days on Succeeding 'Normal' Carbohydrate Diet	
			High	'Normal'	High	'Normal'	0	3	6	10	1	7
1	F	15	390	253	7	4	66	90	108	104	57	51
2	F	14	412	244	8	5	40	178	97	166	66	66
3	M	12	446	245	9	5	27	50	48	41	—	28
4	F	8	296	188	14	9	76	293	113	—	41	30
5	F	6	250	158	15	9	52	94	118	—	47	46
6*	M	8	333	—	14	—	34	115	82	—	—	—

*Normolipidaemic child.

TABLE II

Triglyceride in Lipoprotein Fractions Before and After 3 Days on High-carbohydrate Diet

Case No.	Triglyceride in Lipoprotein Fractions: Very Low Density Lipoproteins* (mg./100 ml. serum)		Low Density Lipoproteins (mg./100 ml. serum)		High Density Lipoproteins (mg./100 ml. serum)	
	Before	On Diet	Before	On Diet	Before	On Diet
1	38	69†	19	27†	9	12†
2	13	132	20	35	7	11
3	8	23	9	14	10	14
4	47	230	21	46	8	17
5	23	60	20	25	9	9
6‡	17	85	11	17	6	13

*Calculated by subtraction of low density and high density triglyceride from whole serum triglyceride.
†6 days on diet.
‡Normolipidaemic child.

red in VLDL, though small increases were also seen in the LDL and HDL fractions. A pre-β lipoprotein band appeared on paper electrophoresis after high-carbohydrate feeding in the normolipidaemic child (Fig.); in the hypercholesterolaemic children, in whom β-lipoprotein was increased, a pre-β band was not seen owing to the breadth of the β band.

Changes in the lipid composition of the lipoprotein fractions in the normolipidaemic child and in one hypercholesterolaemic child are shown in Table III. In both cases the proportion of triglyceride relative to total cholesterol plus phospholipid was increased in all fractions after high-carbohydrate feeding. In the LDL and HDL fractions the amount of total cholesterol and phospholipid decreased.

Changes in the *percentage fatty acid composition of triglyceride* in whole serum and in individual lipoproteins are shown in Table IV. After 3 days on the diet an increase in the percentage of palmitoleic acid and a decrease in the percentage of linoleic acid occurred in every child and were found in all the lipoproteins. No consistent changes were seen in the percentages of the other fatty acids;

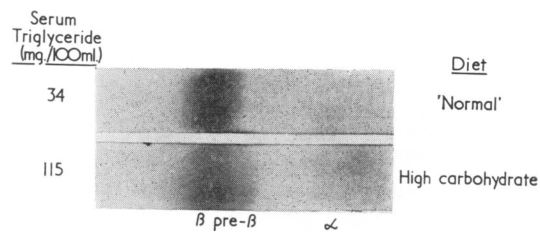

FIG.—*Effect of high-carbohydrate diet on lipoprotein electrophoresis of normolipidaemic child (Case 6).*

TABLE III

Lipid Composition of Lipoprotein Fractions Before and On High-carbohydrate Diet

Lipoprotein Fraction	Case No.	Total Cholesterol (mg./100 ml. serum)		Phospholipid (mg./100 ml. serum)		Triglyceride† (mg./100 ml. serum)		$\frac{TG}{TC + PL + TG} \times 100$	
		Before	On Diet	Before	On Diet	Before	On Diet	Before %	On Diet %
Very low density	1	49	46	38	39	37	50	30	37
	6*	12	34	12	44	15	77	38	50
Low density	1	282	209	133	125	19	27	4	7
	6*	120	103	70	61	11	17	5	9
High density	1	55	41	81	75	9	12	6	9
	6*	52	31	109	74	6	13	4	11

TG = triglyceride, TC = total cholesterol, PL = phospholipid.
*Normolipidaemic child.
†Estimated directly in VLDL fraction.

TAB

Major Triglyceride Fatty Acids (g./100 g. total fatty acids: means a

Fatty Acid	Whole Serum			Very Low Density Lipoproteins		
	Before	On Diet	Difference	Before	On Diet	Difference
C 14 : 0	2·7 (2·0–4·5)	3·6 (2·1–4·6)	+0·9 (−0·7 to +2·5)	3·9 (2·2–5·9)	3·3 (2·4–4·2)	−0·6 (−1·7 to +0·6
C 16 : 0	28·4 (25·3–32·0)	31·2 (26·3–37·2)	+2·8 (−1·8 to +7·9)	28·9 (28·0–31·1)	31·3 (27·2–37·4)	+2·4 (−1·1 to +9·4
C 16 : 1	5·5 (4·6–6·0)	9·0 (6·9–11·8)	+3·5 (+1·3 to +6·3)	4·8 (4·0–5·9)	9·1 (7·3–10·8)	+4·3 (+3·3 to +5·7
C 18 : 0	5·3 (3·7–6·8)	3·7 (2·9–4·6)	−1·6 (−3·4 to +0·9)	5·8 (4·1–6·6)	3·6 (2·1–5·0)	−2·2 (−2·9 to −1·0
C 18 : 1	46·3 (43·2–49·3)	44·9 (36·0–48·1)	−1·4 (−7·2 to +2·8)	44·5 (41·5–47·8)	45·9 (38·9–50·6)	+1·4 (−7·1 to +5·4
C 18 : 2	12·4 (6·1–16·1)	8·7 (4·6–11·0)	−3·7 (−6·2 to −1·5)	11·6 (9·1–14·0)	7·2 (4·2–9·6)	−4·4 (−5·6 to −2·7

the percentage of palmitic acid increased in 3 children and that of oleic acid decreased in 1 child.

Changes in the *absolute concentration of the triglyceride fatty acids* are shown in Table V. With the increase in serum triglyceride on high-carbohydrate feeding, all the fatty acids, including linoleic acid, increased in concentration.

Discussion

Our finding that diets low in total fat and high in carbohydrate lead to increases in serum triglyceride in children with familial hypercholesterolaemia agrees with observations made by Kuo and Bassett (1965) in 2 boys aged 9 and 18 years, and indicates that attention should be given to the carbohydrate as well as to the fat content of diets used to treat this condition. Though it has been suggested that the hypertriglyceridaemic effect of high-carbohydrate diets may not be sustained (Antonis and Bersohn, 1961; Lees and Fredrickson, 1965), our experience (unpublished) with one hypercholesterolaemic child who was treated with a low-fat, high-carbohydrate diet alone showed that serum triglyceride levels were still raised 4 months after starting treatment. In our short-term studies a prompt reduction in serum triglyceride levels occurred when carbohydrate was replaced by fat in the form of corn oil, and our experience with the long-term use of diets low in saturated fat and supplemented with corn oil has shown that serum triglyceride levels remain normal.

The initial serum triglyceride levels of our hypercholesterolaemic children were normal, and the rise in triglyceride on high-carbohydrate feeding may represent the normal response to such diets in childhood. Our child with normal lipoproteins showed a similar response, and the findings are also similar to those reported by Lees and Fredrickson (1965) in healthy young adults. Our studies of the individual lipoproteins show that the increase in triglyceride is not limited to the very low density

TABI

Major Triglyceride Fatty Acids (mg./100 ml. serum: means and rang

Fatty Acid	Whole Serum			Very Low Density Lipoproteins		
	Before	On Diet	Difference	Before	On Diet	Difference
C 14 : 0	1 (1–2)	6 (2–12)	+5 (+1 to +10)	<1 (<1–1)	3 (1–4)	+3 (+1 to +3)
C 16 : 0	13 (7–24)	47 (14–109)	+34 (+7 to +85)	4 (3–5)	19 (5–41)	+15 (+2 to +36)
C 16 : 1	2 (2–4)	15 (4–37)	+13 (+2 to +33)	<1 (<1–1)	6 (1–11)	+6 (+1 to +10)
C 18 : 0	3 (2–5)	5 (1–10)	+2 (−1 to +6)	1 (<1–1)	2 (1–3)	+1 (0 to +2)
C 18 : 1	23 (12–33)	59 (24–105)	+36 (+12 to +72)	5 (4–8)	26 (10–42)	+21 (+5 to +34)
C 18 : 2	6 (2–9)	12 (5–20)	+6 (+2 to +11)	1 (1–2)	4 (2–7)	+3 (+1 to +5)

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nge) in 6 Children before and after 3 days on High-carbohydrate Diet

Low Density Lipoproteins			High Density Lipoproteins		
Before	On Diet	Difference	Before	On Diet	Difference
3·4	3·2	−0·2	3·1	4·0	+0·9
(1·9–6·3)	(2·2–4·2)	(−2·1 to +1·6)	(1·8–4·9)	(3·2–5·0)	(−1·1 to +3·2)
28·4	30·2	+1·8	27·9	29·8	+1·9
(25·1–33·8)	(28·4–34·9)	(−5·3 to +8·5)	(25·4–29·2)	(25·6–35·4)	(−2·5 to +7·4)
5·2	8·8	+3·6	5·9	9·2	+3·3
(4·0–6·5)	(7·4–10·9)	(+0·9 to +6·7)	(4·9–8·1)	(6·4–11·4)	(+1·0 to +6·1)
6·2	4·3	−1·9	5·8	4·8	−1·0
(4·3–9·8)	(3·7–5·0)	(−4·8 to +0·2)	(4·8–6·9)	(3·0–7·5)	(−2·4 to +2·7)
44·8	45·7	+0·9	45·8	43·2	−2·6
(35·9–50·7)	(40·5–51·2)	(−8·0 to +7·9)	(43·7–48·2)	(40·0–48·6)	(−7·9 to +4·9)
12·0	8·6	−3·4	11·1	9·4	−1·7
(6·0–16·7)	(3·3–12·8)	(−6·0 to +0·4)	(6·5–15·3)	(4·2–13·2)	(−4·5 to +1·7)

fraction, and that the lipid composition of all fractions is altered by the incorporation of additional triglyceride.

Triglyceride synthesized from carbohydrate in adipose tissue consists mainly of palmitic, palmitoleic, and oleic acids (Longenecker, 1939; Hirsch *et al.*, 1960; Kuo, Huang, and Bassett, 1961). However, whereas palmitoleic acid and to a lesser extent palmitic acid show percentage increases after high-carbohydrate feeding, oleic acid, which is normally the major fatty acid of triglyceride, shows a small percentage decrease (Longenecker, 1939). In plasma triglyceride Kuo and Bassett (1965) have made similar observations; percentage increases in palmitoleic and palmitic acids and a percentage decrease in oleic acid occurred in a group of 5 hypertriglyceridaemic patients and in 2 subjects with familial hypercholesterolaemia receiving high-sucrose diets. Our findings in relation to changes in the percentage composition of triglyceride fatty acids are in agreement when the mean values for the group are considered, though an increase in palmitic acid and decrease in oleic acid did not occur in all the children. When we determined absolute quantities of fatty acids we found that, with increases in serum triglyceride on high-carbohydrate feeding, all fatty acids increased in concentration. Oleic, palmitic, and palmitoleic acids together were responsible for most of the increase in total triglyceride concentration. Nevertheless, the increase of the other fatty acids, including linoleic acid, indicates either that these fatty acids can also be formed from carbohydrate and/or that some of the increase in triglyceride during high-carbohydrate feeding is due to accelerated esterification of plasma non-esterified fatty acids.

The fact that the concentration of all fatty acids in serum triglyceride is increased during lipogenesis does not invalidate the use of the percentage composition of triglyceride fatty acids as an indicator of enhanced lipogenesis. In our 6 subjects the

: 6 Children before and after 3 days on High-carbohydrate Diet

Low Density Lipoproteins			High Density Lipoproteins		
Before	On Diet	Difference	Before	On Diet	Difference
<1	1	+1	<1	<1	0
(<1–1)	(<1–1)	(0 to +1)	(<1)	(<1–1)	(0 to +1)
5	8	+3	2	4	+2
(3–6)	(4–16)	(+1 to +11)	(2-3)	(2–6)	(0 to +4)
1	3	+2	<1	1	+1
(1)	(1–5)	(0 to +4)	(<1–1)	(1–2)	(+1 to +2)
1	1	0	<1	<1	0
(1)	(1–2)	(0 to +1)	(<1–1)	(<1–1)	(0)
7	12	+5	4	5	+1
(3–10)	(6–19)	(+2 to +9)	(3–5)	(4–6)	(0 to +3)
2	2	0	1	1	0
(1–4)	(1–4)	(−1 to 0)	(<1–1)	(1–2)	(0 to +1)

changes consistently found were an increase in the percentage of palmitoleic acid and a decrease in the percentage of linoleic acid. Because the values for linoleic acid may also be much affected by the amount and type of dietary fat absorbed, we would regard an increased percentage of palmitoleic acid as the most reliable single indicator of accelerated lipogenesis due to high-carbohydrate feeding.

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